TOG PROTEINS ARE SPATIALLY REGULATED BY RAC-GSK3β TO CONTROL INTERPHASE MICROTUBULE DYNAMICS

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ABSTRACT

Kathryn P. Trogden: TOG Proteins are spatially regulated by Rac-GSK3β to control interphase microtubule dynamics
(Under the direction of Stephen L. Rogers)

Within a cell, the ends of individual microtubules switch between three different phases: growth, shrinkage and pause without affecting the total mass of microtubule polymer. This inherent property of microtubules is termed dynamic instability. During interphase, microtubule dynamics are regulated by a class of proteins that interact primarily with the plus end of the microtubule (+TIPs). How these proteins are regulated, either through interactions with each other or through signaling pathways in the cell are poorly understood. Two +TIP protein families, the XMAP215 family, known as Mini spindles (Msps) in *Drosophila* and the CLASP family, known as Orbit, contain arrays of tubulin binding TOG domains. We show that in *Drosophila* S2 cells Orbit is phosphorylated by the kinase GSK3β, similar to what has been reported for mammalian CLASPs. At the periphery of the cell, GSK3β is inhibited by the Rho GTPase Rac and Orbit is no longer phosphorylated. Orbit is then able to interact with Msps through their C-termini, allowing Msps to bind to the lattice. Msps lattice binding is important for proper microtubule dynamics as mutations that disrupt this interaction lead to aberrant dynamics. This interaction requires the scaffolding protein Sentin to bring the two proteins into contact at the periphery of the cell. Further work has shown a role for another Rac effector kinase, PAK, in this pathway. This interaction may also be important for migration in *Drosophila* cells. Depletion of +TIPs leads to changes in cell movement, indicating that microtubule dynamics may play a role in motility.

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PREFACE

Chapter 2 is a manuscript in review at MBoC. I performed all of the experiments and anaylsis and drafted the figures. Stephen Rogers and I designed experiments and drafted the manuscript.

Trogden, K.P., and Rogers, S.L. (2015). Spatial regulation of *Drosophila* Mini-spindles by Rac and GSK3β requires its interaction with Orbit. Mol Biol Cell. In review.

Chapter 3 describes an ongoing project that is a collaborative effort between Stephen Rogers and me to further explore the pathway described in Chapter 2. I performed all of the experiments and anaylsis.

Chapter 4 describes an ongoing project to study the role of the pathway decribed in Chapter 2 in cell migration. All expirements and anaylsis were performed by me. Experimental design was overseen by Derek Applewhite and Steve Rogers.

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LIST OF ABBREVIATIONS AND SYMBOLS

CH	calponin homology
ConA	concanavalin A
CA	constitutively active
$CuSO_4$	copper sulfate
CLASP	Cytoplasmic linker associated protein
CLIP	Cytoplasmic linker protein
EB	End binding
FBS	fetal bovine serum
GFP	green fluorescent protein
GEF	guanine nucleotide exchange factor
MAP	microtubule associated protein
MTOC	microtubule organizing center
+TIP	microtubule plus end interacting protein
Mtl	Mig2-like protein
Msps	Mini spindles
PAK	p21 activated kinase
PBS	phosphate-buffered saline
RNAi	RNA interference
TIRF	total internal reflection fluorescence
μm	micrometer